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4. **Title of the invention** — INSECT CONTROL SYSTEM

5. **Name of your agent** *(if you have one)* — D YOUNG & CO

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Insect Control System

The present invention relates to a method for controlling populations of insects. In particular, the invention relates to a method for controlling a population of insects by transforming at least a subset of the population with a gene encoding one constituent of an enzyme/prodrug system and a promoter capable of driving the coding sequence in the target insects in a sex-specific manner.

Insects are responsible to widespread damage to crops world-wide, with enormous concomitant economic consequences. In order to try to reduce insect-inflicted damage, resources have been devoted to the development and deployment of insecticides, which control insect populations by killing target insects. Although insecticides are in many cases effective, they are known to be toxic to life-forms other than target insects, which has important environmental consequences. Moreover, they are expensive both to produce and to apply to crop-growing areas.

Attempts have moreover been made to control insects by biological means. Methods currently employed to control the populations of certain members of the dipteran class include the release of sterile males.

For example, as set forth in US patent 5, 840, 865, the Mediterranean fruit fly (Medfly) *Ceratitis* (*C.*) *capitata* is a major agricultural pest for many fruit species that is geographically widespread in tropical and temperate regions. The Medfly has been introduced relatively recently into the New World, and appears to be spreading rapidly, threatening fruit producing areas in North America (Carey, J. R., Science 253: 1369 (1991)). Since the mid 1970's, the sterile insect technique has been used successfully for Medfly eradication and control. This method relies on the decrease in or collapse of fly populations following releases of large numbers of sterile insects over infested areas, and offers an environmentally attractive alternative to massive spraying with insecticides (Knipling, E. F., Science 130: 902 (1959)).

Although the use of sterile male insects slows Medfly population growth and may lead to its collapse, it does not lead to actual destruction of female insects, which are responsible for crop damage. Moreover, since the sterile males do not reproduce, the method requires repeated releases of sterile males into the environment.

There therefore remains a need for a control technique for Medfly and other insect pests which selectively destroys female or male insects but which is environmentally more acceptable than the mass spraying of toxic insecticides.

Furthermore, many human and veterinary health issues are associated with the spread of disease by insects. Examples include mosquitoes, tse-tse flies and the common housefly. Control of insect populations which endanger human or animal health is thus also desirable.

Enzyme/prodrug systems are known in the art. A prodrug is a drug, often a potentially toxic drug, which is selectively activated (i.e. rendered toxic) by the action of an enzyme. Enzyme/prodrug systems rely on the delivery of an enzyme to target cells or organisms, before administration of the prodrug. Only target cells or organisms which express the enzyme will be affected by the prodrug. One common enzyme/prodrug system is the 5-fluorouracil/cytosine deaminase system, in which the non-toxic precursor 5-fluorocytosine (5-FC) is converted to the cytotoxic drug 5-fluorouracil (5-FU) by the action of cytosine deaminase (see Austin & Huber, (1993) Mol. Pharmacol. 43:380-387).

Prodrugs, as well as being inactive in organisms or cells that cannot convert them, may also have other advantages, such as improved lipid solubility and/or chemical stability.

Proinsecticides, or prodrug toxins that have been designed to be convertible only in certain insects, have been prepared by selective derivatisation of the final toxin, especially in the case of organophosphates and carbamates. Examples include precursors of acetylcholinesterase inhibitors, such as parathion and profenofos, which are oxidised into active insecticides by enzymes of the cytochrome P-450 family. However, application of proinsecticides to insect populations only kills the insects which naturally express the

enzyme. To render insect pests which do not naturally express the converting enzyme susceptible requires transfer of the gene encoding the converting enzyme into the host and a means of transfer of the gene in an environmentally acceptable manner within the target insect population

Summary of the Invention

In a first aspect of the invention, there is provided a method for controlling a population of target insects, comprising:

a) providing a gene comprising a coding sequence encoding one constituent of an enzyme/prodrug system and a promoter capable of driving the coding sequence in the target insects in a sex-specific manner;

b) transforming at least part of the population of target insects with the gene, and allowing the transposable elements to spread within the target insect population; and

c) administering to the population of target insects the remaining constituent(s) of the enzyme/prodrug system.

As used herein, a "population of target insects" refers to any group of insects, whether delimited along species or geographical lines, or both, which it is desired to control. For example, a population of insects may be refer to a given species of insect which infests a particular crop in a given geographical area. Alternatively, it may refer to all insects infesting any crop in a geographical area, or a given species without reference to any geographical limitation, or a population of insects which is responsible for a human or veterinary health problem, such as the spread of malaria. Target insects are the individual members of the population of insects.

"Control" refers to the limitation, prevention or reduction of growth of the insect population. Preferably, this is achieved by killing target insects. Advantageously, the population of insects is eliminated.

"Gene", as used herein, refers to a nucleic acid, usually DNA, which comprises a sequence of nucleotides which encode a protein or polypeptide and the sequences required to transcribe the nucleotide sequence in a suitable host cell. The nucleotide sequence is referred to herein as a "coding sequence" and the sequences required for transcription are referred to as "promoters".

The coding sequence encodes one constituent of an enzyme/prodrug system. The constituent may be any one or more parts of the system, as long as it is not in itself sufficient to produce the active drug from the prodrug. Thus, the constituent is preferably the enzyme which is responsible for prodrug activation. Alternatively, it may be the prodrug itself. The remaining constituent(s) of the enzyme/prodrug system are administered separately, thus killing the target insects which express the coding sequence according to the invention.

A feature of the present invention is that the promoter used to drive transcription of the coding sequence is active in a sex-specific manner. This means that the coding sequence is expressed substantially only in one sex of the target insects, be it male or female. As used herein, "substantially only" advantageously refers to a selectivity for a given sex of 70:30, 80:20, 90:10 or preferably 100:0.

Preferably, wherein the gene is used to transform male target insects. This is especially preferred in cases where the promoter which drives the coding sequence is active to express the coding sequence only in female target insects. This allows the selective destruction of female insects, whilst maintaining a population of male insects which are able to pass on the gene comprising the coding sequence whilst remaining unaffected by the prodrug. This is highly advantageous where the female is responsible for spreading damage or disease.

In cases where the male is responsible for spreading damage or disease, the technique may be reversed, such that a coding sequence only functional in male insects is spread by carrier females.

The invention furthermore provides an insect of a given sex, which insect has been transformed with a gene comprising a coding sequence encoding one constituent of an enzyme/prodrug system and a promoter capable of driving the coding sequence substantially only in insects of the opposite sex.

In a further aspect, the invention provides a vector which is capable of transforming a target insect cell, which vector comprises a gene comprising a coding sequence encoding one constituent of an enzyme/prodrug system and a promoter capable of driving the coding sequence in target insects in a sex-specific manner.

Detailed Description of the Invention

The present invention is applicable to the control of any insect population, comprising any target insects, whether the population is homogenous or heterogeneous. For example, insects which are controlled using the present invention include:

Bactrocera oleae (olive fly) - affects olive crops
Bactrocera orientalis (oriental fruit fly) - affects many fruit crops
Heliothis armigera (cotton bollworm) - affects cotton
Trichoplusa ni (cabbage looper) - affects cabbages
Manduca sexta (tobacco hornworm) - affects tobacco
Lobesia botrana (grapevine moth) - affects grapes
Anopheles gambiae (mosquito) - carries malaria
Aedes aegypti (yellow fever mosquito) - yellow fever
Glossina morsitans (tse-tse fly) African trypanosomiasis (sleeping sickness)
Simulium sp. (black fly) onchocerciacis (river blindness)
Phlebotomus sp. (sand fly) visceral leishmaniasis (kala-azar)
Musca domestica (house fly) many bacterial infections.

Preferred, however, are insect populations which include or consist of the Mediterranean fruitfly, *C. capitata* (Medfly).

Genes useful in the practice of the invention, as well as vectors encoding them, may be prepared according to standard approaches used in molecular biology. See, for example, Sambrook *et al.*, Molecular Cloning, A Laboratory Manual (1989) and Ausubel *et al.*, Short Protocols in Molecular Biology (1999) 4th Ed, John Wiley & Sons, Inc.

Preferably, the coding sequence encodes the esterase/amidase enzyme which converts DPX-JW062 into its active metabolite (Wing *et al.*, (1998) Arch Insect Biochem Physiol 37:91-103). The pro-insecticide is, in this aspect of the invention, DPX-JW062 which is obtainable from Du Pont.

Alternatively, the coding sequence encodes an enzyme of the cytochrome P-450 family. Although most insects possess cytochrome P-450 family enzymes, the efficiency of bioconversion of proinsecticides converted by such enzymes may be increased by transforming the insect with a more efficient and/or overexpressed enzyme, allowing a decrease in the effective dose of the proinsecticide. Suitable proinsecticides in this aspect of the invention include organophosphates and carbamates. For example, the proinsecticide may be an acetylcholinesterase inhibitor such as parathion and profenofos.

According to a further embodiment, the prodrug is p-hydroxyaniline mustard glucuronide, which is converted to the toxin p-hydroxyaniline mustard by the converting enzyme β-glucuronidase, which must be located in the extracellular space (Cheng *et al.*, (1999) Biochem. Pharmacol. 58:325-328). Preferably, therefore, the coding sequence encodes *E. coli* β-glucuronidase.

Preferably, the coding sequence encodes cytosine deaminase, in which case the proinsecticide is 5-FC.

Sex-specific promoters suitable for driving a coding sequence as set forth above are available in the art. In a preferred embodiment, vitellogenin genes encoding yolk proteins may be used as a source of suitable promoters. In the case where the target insects are Medfly (*C. capitata*), the VG1 and VG2 promoters may be used (Rina and Savakis, (1991) Genetics 127:769-80).

The transformation of target insects may be performed using any suitable technique for preparing transgenic insects. Such techniques include DNA transfection, the use of viral vectors, and the use of episomal vectors which are transmitted through germ cells. Advantageously, the transformation is carried out using a suitable vector. The invention thus provides a vector which is capable of transforming a target insect cell, which vector comprises a gene comprising a coding sequence encoding one constituent of an enzyme/prodrug system and a promoter capable of driving the coding sequence in target insects in a sex-specific manner.

Preferably, the vector is a transposon or transposable element. The invention accordingly provides a method as set forth above, wherein the transformation step comprises the steps of:

a) providing a transposable element which encodes a transposase protein which is active in the target insects;

b) modifying the transposable element by inserting the gene; and

c) transforming the target insects with the modified transposable element.

Transposons are genetic elements which are capable of "jumping" or transposing from one position to another within the genome of a species. They are widely distributed amongst animals, including insects.

Transposons are active within their host species due to the activity of a transposase protein encoded by the elements themselves. Advances in the understanding of the mechanisms of transposition have resulted in the development of genetic tools based on transposons which can be used for gene transfer.

Any transposable element active in the desired target insect may be used. Preferably, however, the transposable element is selected from the group consisting of *Minos*, *mariner*, *Hermes* and *piggyBac*.

Minos is a transposable element which is active in Medfly. It is described in US patent 5,840,865, which is incorporated herein by reference in its entirety. The use of *Minos* to transform insects is described in the foregoing US patent.

Mariner is a transposon originally isolated from *Drosophila*, but since discovered in several invertebrate and vertebrate species. The use of *mariner* to transform organisms is described in International patent application WO99/09817.

Hermes is derived from the common housefly. Its use in creating transgenic insects is described in US patent 5,614,398, incorporated herein by reference in its entirety.

PiggyBac is a transposon derived from the baculovirus host *Trichplusia ni*. Its use for germ-line transformation of Medfly has been described by Handler *et al.*, (1998) PNAS (USA) 95:7520-5.

According to the present invention, insects are generated which can be released into populations of insects and which will interbreed with those populations to produce target insects which are susceptible to a proinsecticide. The invention thus relates to an insect of a given sex, which insect has been transformed with a gene comprising a coding sequence encoding one constituent of an enzyme/prodrug system and a promoter capable of driving the coding sequence substantially only in insects of the opposite sex. Preferably, the insect is a male insect. Advantageously, the insect is a Medfly.

The invention is further described, for illustration only, in the following example.

Example

Using standard methods, a genetic construct is prepared bearing the *C. capitata* VG1 promoter operably linked to a cDNA encoding *E. coli* cytosine deaminase. The cytosine deaminase gene, when introduced into insect cells, confers sensitivity to the prodrug 5-FC.

The construct is inserted into the *Minos* transposon using conventional techniques and, in addition, those described in US 5, 840, 865. The modified transposon is then used to transform Medfly according to standard transgenesis procedures.

Transgenic flies carrying the modified transposon are bred to create transgenic strains that express the cytosine deaminase enzyme. Characterisation for cytosine deaminase activity shows that the enzyme is expressed at a high level selectively in females. Moreover, cytosine deaminase expression is correlated with susceptibility to the prodrug 5-FC administered in food to the flies.

A highly susceptible strain is bred and male flies isolated. Introduction of male flies into a population of non-transgenic flies results in rapid transfer of the transgene. Female flies which are born inheriting the transgene are susceptible to 5-FC, whilst male files remain resistant.

CLAIMS

1. A method for controlling a population of target insects, comprising:

a) providing a gene comprising a coding sequence encoding one constituent of an enzyme/prodrug system and a promoter capable of driving the coding sequence in the target insects in a sex-specific manner;

b) transforming at least part of the population of target insects with the gene, and allowing the transposable elements to spread within the target insect population; and

c) administering to the population of target insects the remaining constituent(s) of the enzyme/prodrug system.

2. A method according to claim 1, wherein the promoter is active to express the coding sequence in male target insects.

3. A method according to claim 1 or claim 2, wherein the promoter is active to express the coding sequence only in female target insects.

4. A method according to any preceding claim, wherein the coding sequence encodes the esterase/amidase enzyme which converts DPX-JW062 into its active metabolite.

5. A method according to any preceding claim, wherein the coding sequence encodes a cytochrome P-450 enzyme.

6. A method according to any preceding claim, wherein the coding sequence encodes cytosine deaminase.

7. A method according to any preceding claim, wherein the coding sequence encodes β-glucuronidase.

8. A method according to claim 4, wherein the enzyme/prodrug system further comprises DPX-JW062.

9. A method according to claim 5, wherein the enzyme/prodrug system further comprises an organophosphate proinsecticide.

10. A method according to claim 6, wherein the enzyme/prodrug system further comprises 5-FC.

11. A method according to claim 6, wherein the enzyme/prodrug system further comprises p-hydroxyaniline mustard glucuronide.

12. A method according to any preceding claim, wherein the transformation step comprises the steps of

a) providing a transposable element which encodes a transposase protein which is active in the target insects;

b) modifying the transposable element by inserting the gene; and

c) transforming the target insects with the modified transposable element.

13. A method according to claim 12, wherein the transposable element is selected from the group consisting of *Minos*, *mariner*, *Hermes* and *piggyBac*.

14. A method according to any preceding claim wherein the target insect is *Ceratitis capitata*.

15. A method according to claim 14, wherein the promoter is a *Ceratitis capitata* yolk protein gene promoter.

16. An insect of a given sex, which insect has been transformed with a gene comprising a coding sequence encoding one constituent of an enzyme/prodrug system and a promoter capable of driving the coding sequence substantially only in insects of the opposite sex.

17. An insect according to claim 16 which is a male insect.

18. A vector which is capable of transforming a target insect cell, which vector comprises a gene comprising a coding sequence encoding one constituent of an enzyme/prodrug system and a promoter capable of driving the coding sequence in target insects in a sex-specific manner.

19. A vector according to claim 18 which is a transposon.

20. A transposon according to claim 19 which is *Minos*.

Abstract

The invention relates to a method for controlling a population of target insects, comprising: a) providing a gene comprising a coding sequence encoding one constituent of an enzyme/prodrug system and a promoter capable of driving the coding sequence in the target insects in a sex-specific manner; b) transforming at least part of the population of target insects with the gene, and allowing the transposable elements to spread within the target insect population; and c) administering to the population of target insects the remaining constituent(s) of the enzyme/prodrug system.